

**CLAIMS:**

1. A purified preparation of human undifferentiated embryonic stem cells capable of proliferation *in vitro*.
2. A purified preparation of human embryonic stem cells according to claim 1 capable of maintaining an undifferentiated state when cultured under conditions which do not induce extra embryonic differentiation and cell death.
3. A purified preparation of human embryonic stem cells according to claim 2 wherein the condition includes cultivating the cells on a fibroblast feeder layer.
4. A purified preparation of human embryonic stem cells according to any one of claims 1 to 3 and capable of differentiation *in vitro* under differentiating conditions.
5. A purified preparation of human embryonic stem cells according to claim 4 wherein the stem cell is capable of differentiation into a somatic cell selected from the group including a committed progenitor cell capable of self renewal and further differentiation into one or several types of mature cell, or a mature differentiated cell.
6. An undifferentiated human embryonic stem cell wherein the cell is immunoreactive with markers for human pluripotent stem cells including SSEA-4, GCTM-2 antigen, and TRA 1-60.
7. An undifferentiated human embryonic stem cell according to claim 6 wherein the cell expresses Oct-4.

8. A method of preparing undifferentiated human embryonic stem cells, said method including:

obtaining an *in vitro* fertilised human embryo and growing the embryo to a blastocyst stage of development;

5 removing inner cells mass (ICM) cells from the embryo; \_

culturing ICM cells under conditions which do not induce extraembryonic differentiation and cell death; and promote proliferation of undifferentiated stem cells; and

recovering stem cells.

10

9. A method of preparing undifferentiated human embryonic stem cells, said method including:

obtaining an *in vitro* fertilised human embryo and growing the embryo to a blastocyst stage of development;

15 removing inner cell mass (ICM) cells from the embryo;

culturing ICM cells on a fibroblast feeder layer to obtain proliferation of undifferentiated stem cells; and

recovering stem cells from the feeder layer.

20 10. A method according to claim ~~9~~ wherein the fibroblast feeder layer is a mouse and/or human fibroblast feeder layer.

11. A method according to claim ~~10~~ wherein the fibroblast feeder layer comprises embryonic fibroblasts.

25

12. A method according to any one of claims ~~9~~ to 11 wherein the fibroblasts are tested for their ability to promote embryonic stem cell growth and to limit extraembryonic differentiation.

30 13. A method according to claim 12 wherein the fibroblast cell strain is highly suitable for the promotion of embryonic stem cell growth and the inhibition of extraembryonic differentiation.

00445154-1099

14. A method according to claim 13 wherein the fibroblast cell strain is derived from the inbred mouse strains 129/Sv, CBA or the cross of 129/Sv and C57/Bl6.

5

15. A method according to any one of the claims 7 to 14 wherein the fibroblast express recombinant membrane bound factors essential for human pluripotent stem cell renewal including human multipotent stem cell factor.

10

16. A method according to any one of claims 8 to 15 further including a preliminary treatment prior to removal of ICM cells, said treatment including:

treating the embryo to dislodge the trophectoderm of the embryo or a portion thereof;

15

washing the embryo with a G2.2 or S2 (Scandinavian-2) medium to dislodge the trophectoderm or a portion thereof; and  
obtaining inner cell mass cells of the embryo.

17. A method according to any one of claims 8 to 16 further including the steps of:

20

replating the stem cells from the fibroblast feeder layer onto another fibroblast feeder layer; and

culturing the stem cells for a period sufficient to obtain proliferation of morphologically undifferentiated stem cells.

25

18. An undifferentiated cell produced by the method according to any one of claims 8 to 17.

Sub B4

19. A method according to any one of claims 8 to 17 further including the step of growing cells under culture conditions that induce somatic differentiation, and wherein said conditions do not permit continued stem cell renewal but do not kill stem cells or induce their unidirectional differentiation into extraembryonic lineages.

30

20. A method according to claim 19 wherein the condition includes prolonged cultivation of the undifferentiated stem cells on a differentiation inducing fibroblast feeder layer to induce a differentiated somatic lineage or multiple somatic lineage.

21. A method according to claim 20 wherein the differentiation inducing fibroblast feeder layer is a mouse and/or human fibroblast feeder layer.

22. A method according to claim 20 or 21 wherein the fibroblast feeder layer comprises embryonic fibroblasts.

23. A method according to any one of claims 20 to 22 wherein the fibroblasts are tested for their ability to promote embryonic stem cell growth and to limit extraembryonic differentiation.

24. A method according to any one of claims 19 to 23 wherein the embryonic fibroblasts are prepared and tested for their ability to allow somatic differentiation of embryonic stem cells.

25. A method according to any one of claims 19 to 24 wherein the culture condition includes cultivating the cells for prolonged periods and/or at high density in the presence of a differentiation inducing fibroblast feeder layer to induce somatic differentiation.

26. A method for the isolation of committed progenitor cells from a culture of differentiated cells said method comprising:

preparing a culture of differentiated cells according to any one of claims 19 to 25; and

isolating committed progenitor cells from the culture.

2015-10-14 10:00

27. A differentiated cell produced by the method according to any one of claims 19 to 26.

28. A differentiated cell according to claim 27 which is a somatic cell selected from the group including a committed progenitor cell capable of self renewal or differentiation into one or several somatic lineages, or a fully mature somatic differentiated cell.

29. A cell line HES-1.

30. A cell line HES-2.

31. A fibroblast cell strain which is highly suitable for the promotion of embryonic stem cell growth and the inhibition of extraembryonic differentiation.

32. A fibroblast cell strain according to claim 31 derived from the inbred mouse strains 129/Sv, CBA or the cross of 129/Sv and C57/Bl6.

33. A method of preserving a differentiated or undifferentiated cell wherein the cells undergo vitrification.

34. A method according to claim 33 wherein the vitrification is Open Pulled Straw (OPS) vitrification.

35. A method of preventing and treating a congenital disease, said method including:

obtaining an undifferentiated stem cell according to claim 18;

introducing a genetic modification to the congenital disease; and

inducing differentiation to a somatic cell line capable of transplantation to

a patient in need.

add  $B^6$

[illegible]